Summer 2015

Effect of Altered Cellular Redox Environment on Oncogenic Activity of the Drosophila PRL Protein

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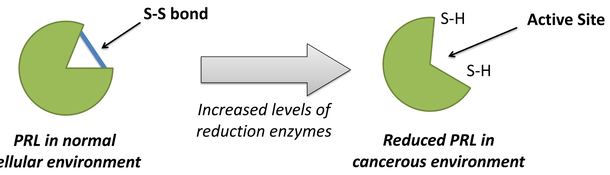
Background

- Cancer is characterized by abnormal cell growth and invasion of other tissues, resulting in the formation of tumors. Tumors can form through mutations in either oncogenes (which promote growth) or tumor suppressors (which inhibit growth).
- Aberrant expression of members of the phosphatase of regenerating liver (PRL) family has been implicated in the progression of several forms of human cancer.[1]
- PRL has been found to function as an oncogene in genetically transformed cells (such as cancerous cells), but in non-transformed cells it functions as a tumor suppressor.[2 3] The mechanisms behind this are still unclear.
- In normal cellular environments, the active site of PRL may be inaccessible due to oxidation of two cysteines to form an intramolecular disulfide bond, preventing activation of oncogenic activity.[4] Genetic transformations in cancerous cells could then reduce PRL by disrupting systems that control redox environment.



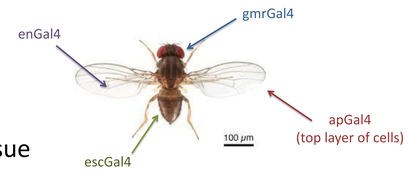
Aims

- Using *Drosophila* (the common fruit fly) as a model organism, overexpress dPRL-1 in wing tissue, eye tissue, and intestinal stem cells.
- Overexpress each of the following proteins that control cellular redox conditions alongside dPRL-1:
  - CncC → inhibits oxidation
  - CncC^{RNAi} → promotes oxidation by inhibiting CncC production
  - Keap1 → promotes oxidation via degradation of CncC
  - Keap1^{RNAi} → inhibits oxidation by inhibiting Keap1 production
- Examine the effect of cellular redox environment on the function of PRL proteins as either tumor suppressors or oncogenes *in vivo.*

Methods

- The UAS/Gal4 driver system was used in order to genetically alter specific tissue types without affecting the rest of the animal. UAS is an enhancer that prevents transcription of the gene of interest until bound by Gal4, a tissue-specific transcription factor. The following drivers were used:

gmrGal4 → eye tissue
escGal4 → gut stem cells
apGal4 → dorsal wing tissue
enGal4 → posterior wing tissue



- By setting several generations of crosses between stock lines of flies with specific genes of interest, flies with the following genotypes were produced:

Experimental genotypes	gmrGal4 apGal4 escGal4 enGal4	+ UAS-Keap1 UAS-Keap1^{RNAi} UAS-CncC UAS-CncC^{RNAi}	+ **UAS-dPRL1**
Control set for altered redox environment	gmrGal4 apGal4 escGal4 enGal4	+ UAS-Keap1 UAS-Keap1^{RNAi} UAS-CncC UAS-CncC^{RNAi}	
Control set for UAS-dPRL-1	gmrGal4 apGal4 escGal4 enGal4	+ **UAS-dPRL1**	

- The eye tissue of flies expressing gmrGal4 was examined via light microscopy and SEM. The wing curvature of flies expressing apGal4 was also examined via light microscopy and SEM.

Results

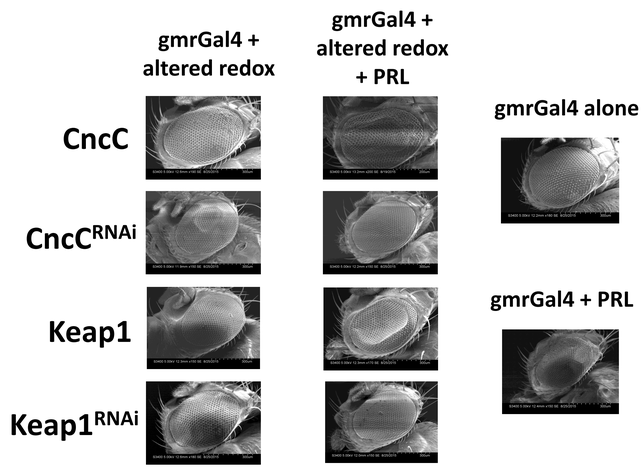


Figure 1. Eye tissue phenotypes of PRL expressed in altered redox environments. Both PRL and each redox-controlling enzyme were overexpressed in the eye tissue of *Drosophila* via the gmrGal4 driver. Animals of each genotype were then imaged via SEM and analyzed. Though difficult to quantify, changing the redox environment alone produced a dramatic phenotype. *Drosophila* with overly reduced cell environments (via CncC and Keap1^{RNAi} overexpression) presented larger eyes, while *Drosophila* with overly oxidized cell environments (via CncC^{RNAi} and Keap1 overexpression) presented smaller eyes. The effects of adding dPRL-1 to these altered eye cells were too minor to draw any significant conclusions. Any collapsed eyes occurred during SEM preparation and are not a result of overexpressed PRL.

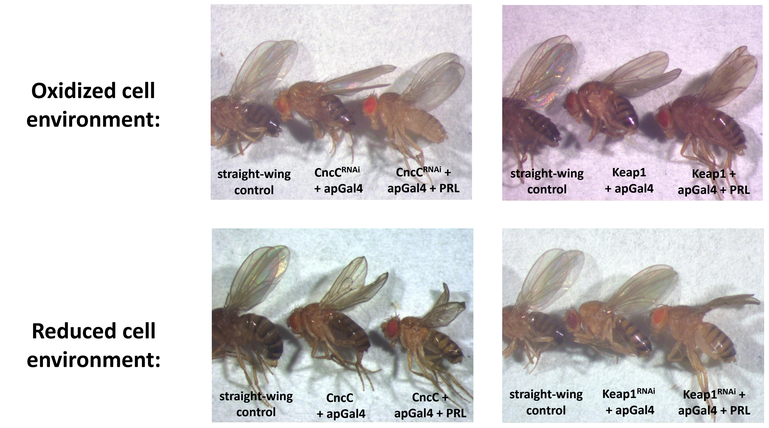


Figure 2. Dorsal wing tissue phenotypes of PRL expressed in altered redox environments. Both PRL and each redox-controlling enzyme were overexpressed in the dorsal wing tissue of *Drosophila* via the apGal4 driver. Animals of each genotype were then imaged via light microscopy and analyzed. Because apGal4 expresses only in the top layer of wing cells, upwards curvature of the wing indicates an undergrowth phenotype, whereas downwards curvature indicates overgrowth. Animals expressing PRL alongside Keap1 and CncC^{RNAi} had wings with a slight upwards curve as compared to their controls, indicating that PRL suppressed growth to a small degree. The most striking undergrowth phenotype was seen in animals expressing CncC and PRL, which presented significantly smaller wings with a distinct upwards curve. The bubbling of the wings also indicates separation between the two layers of wing cells due to this growth suppression. Conversely, the wings of animals expressing Keap1^{RNAi} and PRL had a significant downwards curve, suggesting that PRL here acted as an oncogene.

Discussion

- In gmrGal4 flies, the most significant phenotypes occurred when the cell redox environment was altered, and adding PRL to these altered environments did not result in any quantifiable changes.
- In apGal4 flies, the most significant growth suppression occurred with the CncC + PRL genotype, while Keap1^{RNAi} + PRL resulted in overgrowth. Both CncC and Keap1^{RNAi}, however, inhibit oxidation in the cell. This could suggest that the impact of redox environment on PRL function is much more complex than anticipated.

Continued Work

- Overexpress PRL and altered redox enzymes in gut stem cells via escGal4 and analyze changes in cell proliferation and migration.
- Overexpress PRL and altered redox enzymes in posterior wing tissue via enGal4 in order to quantify changes in tissue growth.
- Note that the above two studies will be the most useful for quantification of results, as they can be used to count cells and accurately quantify tissue size.
- Based on the gmrGal4 results, further examination of the impact of altered redox environment alone on cell growth and proliferation will also be necessary.

Acknowledgements

I would like to thank the University of Puget Sound for funding my research via the Mellam Summer Scholar award. I would also like to thank Leslie Saucedo and the other students in her research lab for their support and collaboration on this project.

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